Clinical Significance of Some Biomarkers in the Detection of Kidney and Bladder Cancers

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Abstract: Background: Screening for biomarkers is the process of finding proteins, genes, and other components (also known as tumor markers or biomarkers) that may provide details about malignancy. Each person's cancer has a particular pattern of biomarkers. Some biomarkers influence how specific cancers develop. The aim of this study was to use biomarkers (C7, CK7. CD117) in the early diagnosis of cancer of the bladder and renal cancer.

Methods: A case-control study with 92 participants, 31 individuals in good health as the control group and 61 patients that correspond to the inclusion standards with an age range from (13 to 92) years, Kits were used to measure CLDN7, CK7. CD117 while Enzyme-Linked Immunosorbent Assay (ELISA).

Results: The current study showed significant differences ($p< 0.05$) in the Age with significant decrease of C7, CK7 and CD117 also showed no significant differences (p 0.057) in the BMI of Renal groups when compared to the case control. Additionally results showed significant differences ($p< 0.05$) in the Age (61.9) with significant decrease of C7, CK7 and CD117 showed significant increase, also showed no significant difference in BMI ($P> 0.285$) of Bladder groups when compared to the case control

Conclusion: The results of the current study demonstrated that the biomarkers C7, CK7, and CD117

can be utilized to diagnose renal cancer due to they all had decreasing concentrations, but only C7 and CK7 have decreasing concentrations in bladder cancer counter to (CD117) concentration is rising.

Key words: RCC, Bladder cancer, C7, CK7, CD117.

1. Introduction

A collection of disorders distinguished by abnormal cell growth and division are referred to collectively by the complex and wide term "cancer." Through a process known as metastasis, it can impact different portions of the body, potentially invading nearby tissues and disseminating to additional areas of the body [1]. Cancer begins when normal cells undergo genetic mutations that cause them to divide and grow uncontrollably, these mutations can be caused by a variety of factors, including genetic predisposition, exposure to certain chemicals or substances (such as tobacco smoke or radiation), certain infections, unhealthy lifestyle choices, and other environmental factor[2]. There are many different types of cancer, including bladder cancer, renal cancer, breast cancer, lung cancer, prostate cancer, colorectal cancer, skin cancer, and many more. Each type of cancer has its own specific characteristics, treatment options, and prognosis [3].

Bladder cancer starts when cells in the bladder mucosa begin to grow out of control. as more tumors develop, they eventually develop a tumor and spread to other regions of a person's body [4].The 10th most prevalent malignancy in the world is Bladder Cancer, and its prevalence is rapidly rising. According to data from the Global Cancer Observatory (GLOBO.CAN), there were 212.536 fatalities and 573.278 new cases of bladder cancer in only 2020, Bladder Cancer accounts for 2% of all new cases of malignancies in GLOBOCAN 2020, with 3,885 new instances of mortality, 9.6 per 100,000 people and 2.4 per 100,000 people, respectively [5]–[7].

Renal cell carcinoma (RCC) is the ninth most frequent kind of cancer to be reported worldwide [8]. Adults with (RCC) have a variety of tumors arising from the renal tubular epithelial cells[9] .RCC, the most common form of adult kidney cancer, is also known as renal adenocarcinoma, hypernephroma, and renal cancer[10]. According to the most recent Global Cancer Statistics report, about 431,288 new cases of kidney cancer were discovered worldwide in just 2020 (figuer1.1), the age standardized ratio (ASR) for this incidence rate was 6.1/100000 for men and 3.2/10000 for women, effective treatment options for accidental RCC have been available for several decades, and they vary from open or robotic-assisted partial/total nephrectomy to minimally invasive percutaneous laparoscopic cryoablation and radiofrequency ablation, individuals with these incidental early-detected small renal tumors now have a better prognosis thanks to the efficacy of these treatment strategies [6], [11] . A collection of biomarkers will be used in this study to evaluate their use in the detection of bladder and Renal cancer. The purpose of the study is to demonstrate that (C7, CK7, CD117) biomarkers can be used in the clinical diagnosis of cancer in place of tissue biopsies.

2. Materials and Methods

61 patients were considered and diagnosed with cancer. In the study, population age ranged from (13-92 years) old. The study also included (31) healthy persons as control. Who visited Al-Basra Oncology Centre in Al-Sadr Teaching Hospital in Basra. All patients in this research were diagnosed by oncologist Al-Basra Oncology Centre in Al-Basra Teaching Hospital and confirmed by all clinical and laboratory investigations. The practical analyses of the study were carried out in the department of Medical Laboratory at STU, Basra. Kits were used Enzyme-Linked Immunosorbent Assay (ELISA) was applied to measure C7, CK7 and CD117.

2.1 Statistical Analysis

Mean and variance (SD) of the data were computed. The t-test (for means) and the chi-square test were used to see if there were any differences between the groups (for frequencies). SPSS for Windows was used to conduct all statistical analyses (version 23, USA). ANOVA was employed for normally distributed data. Example is when $P < 0.05$ was statistically considered significant, while $P > 0.05$ was judged non-significant.

3. Results and Discussion

3.1. Characteristics of studied groups

This study included 92 patients and control [Bladder group N= 30, Renal group N= 31 and Control group N= 31) with an age range from (13 to 92) years, in the group of Bladder total number = 30 (Male = 23 and Female = 7), the Renal group total= 31 (Male= 15 and Female=16) and in the control group number= 31 (Male= 18 and Female=13) figure (1) show the demographic characteristic of study.
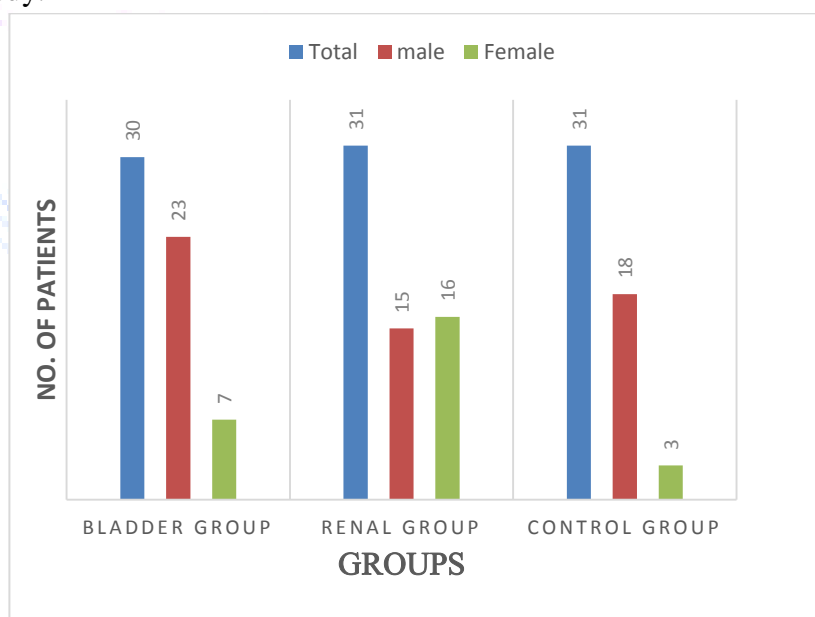

figure (1) demographic characteristic of study

3.2 bladder cancer results

The anthropometric data were illustrated in table (1), The current study was demonstrated significant difference in Age, CLDN7, CK7 and CD117 with no significant difference in BMI.

Table (1): Statistical analysis for Age, BMI, CLDN7, CK7 and CD117 in Bladder group compared to the control group.

Parameter	Bladder group (N=30)	Control group	P

	Mean	SD	(N=31) Mean	SD	*value*
Age (year)	61.9	15.6	50.3	13.1	0.003
BMI (Kg/m^2)	24.6	4.27	26.6	6.05	0.258
CLDN7 serum	30.7	14.9	117	28.1	< 0.001
CLDN7 tissue	33.2	12.7	130	27.2	< 0.001
CK7 serum	1.84	0.931	4.40	1.19	< 0.001
CK7 Tissue	1.67	0.783	4.70	1.19	< 0.001
CD117 serum	104	13.9	43.7	19.4	< 0.001
CD117 tissue	98.9	16.9	47.4	19.9	< 0.001
N: number of cases SD: standard deviation significant at p < 0.05					

the results showed that with age, the risk of bladder cancer increases (P value <0.05), this result agrees with what was mentioned by Wang et al., 2019 and Bermejo et al., 2019 [12], [13]. The probability of developing bladder cancer increases with age due to a combination of factors, including biological changes that occur with aging, environmental exposures, other lifestyle factors and Changes in urinary system function including a decrease in bladder capacity and changes in bladder function, which may increase the risk of bladder cancer. For example, Liu et al find that changes in bladder function may lead to more frequent urinary tract infections, which are a known risk factor for bladder cancer[14].

The results show a statistically significant difference P-value (<0.05) between the bladder group and the control group for CLDN7 (Table 1). the results agree with the results of [15]–[17]. All these studies reported that the CLDN7 expression is decreased in bladder cancer compared to normal bladder tissue. These studies reported that CLDN7 expression is decreased in bladder cancer compared to normal bladder tissue. Maesaka et al reported the lower expression of CLDN7 in bladder cancer may be attributed to epigenetic modifications, which influence the expression of genes without altering the DNA sequence underlying them. For instance, some research has revealed that the frequent epigenetic change of DNA methylation controls the production of CLDN7. DNA methylation may cause the expression of CLDN7 to be down regulated in bladder cancer [18]. Bhat et al find a crucial component in preserving the integrity of epithelial tissues is the creation of tight junctions between cells, which CLDN7 contributes to. The down regulation of CLDN7 expression in bladder cancer may be caused by changes in tight junctions, For example, some studies have suggested that the loss of Ecadherin, a protein that interacts with CLDN7 to form tight junctions, may lead to the down regulation of CLDN7 in bladder cancer [15].

Also, as show in Table (1) a statistically significant difference with a P value (<0.05) between the bladder group and the control group for CK7 as the concentration of CK7 was low in samples of bladder cancer patients compared with control samples. These results agree with [19], [20] . Cytokeratin 7 (CK7) is a protein that is expressed in normal urothelial cells, which are the cells that line the inside of the bladder. However, in some cases of bladder cancer, the expression of CK7 can be reduced or absent [21]. Alshahwan et al find that there are several possible explanations for this. One hypothesis is that the kind of bladder cancer may be connected to the lack of CK7 expression. For instance, CK7 expression is frequently diminished in invasive bladder tumors as opposed to non-invasive tumors, according to several research. Additionally, according to certain studies, certain bladder cancer types may have a worse prognosis if their CK7 expression is low [22]. Another possibility is that the loss of CK7 expression may be related to the degree of differentiation of the tumor cells. Tumor cells that are poorly differentiated (meaning they look very different from normal urothelial cells) may be less likely to express CK7 [23].

Table (1) shows that there is a statistically significant difference P-value (<0.05) between the bladder group and the control group for CD117, where the level of CD117 was clearly high in patients with bladder cancer compared to the control samples, and these results agree with [24], [25]. Wu et al reported that the prognosis of primary bladder was poor even after multimodal treatment and demonstrated high expression of CD117 (c-kit) in tumor cells [26] . CD117 is a protein that is also known as c-kit or stem cell factor receptor. It plays an important role in the regulation of cell growth, differentiation, and survival, CD117 expression has been found to be increased in some cases of bladder cancer [27] . the exact reasons for increased CD117 expression in bladder cancer are not fully understood. However, Hamidfar et al find has suggested that it may be related to certain genetic alterations or mutations, For example, mutations in the c-kit gene, which encodes the CD117 protein, have been found in some bladder cancer cases [25].

Additionally, the result shows no significant different P value (>0.05) for BMI between Bladder and control group also this result agrees with Evers et al., 2020 and Awadalla et al., 2020 [28], [29].

3.3 Renal cancer results

Table (2) contains the anthropometric statistics. The current investigation showed significant differences in age, CLDN7, CK7, and CD117, but not in BMI.

Table (2): Statistical analysis for Age, BMI, CLDN7, CK7 and CD117 in Renal group compared to the control group.

Parameter	Renal group (N=31)		Control group (N=31)		P value
	Mean	SD	Mean	SD	
Age (year)	58.2	16.3	50.3	13.1	0.041
BMI (Kg/m^2)	28.6	6.05	26.6	6.05	0.057
CLDN7 serum	17.2	8.83	117	28.1	<0.001
CLDN7 tissue	12.4	4.90	130	27.2	<0.001

CK7 serum	1.39	0.551	4.40	1.19	<0.001
CK7 Tissue	1.38	0.670	4.70	1.19	<0.001
CD117 serum	7.00	2.08	43.7	19.4	<0.001
CD117 tissue	6.08	1.96	47.4	19.9	<0.001
N: number of cases SD: standard deviation significant at p < 0.05					

The results show a significant difference P value (< 0.05) among RCC groups conformed with control group for Age Table (2). while showed that with age the probability of RCC risk increases, and this agrees with the results of Bermejo et al., 2019; Schouten et al., 2022; Zhu et al., 2022 [12], [30], [31]. The probability of developing RCC increases with age due to a combination of factors, including biological changes that occur with aging, environmental exposures, and other lifestyle factors. As more mutations arise with aging cause increase of develop cancer. Takeshima & Ushijima find the probability of developing RCC is expected to increase with the accumulation of mutations in the genes that control cell growth and division, which are regarded to be the cause of the disease [32]. The ability of immune system to identify and eliminate aberrant cells decreases with aging, which can aid in the growth of cancer , due to the immune system's crucial involvement in recognizing and eliminating kidney cancer cells, this reduction in immune function may be especially important for RCC [33]. Exposure to environmental risk factors like Smoking, exposure to certain chemicals, and obesity are among the environmental factors that are known to increase the chance of developing RCC [34]. A number of lifestyle variables, including nutrition and exercise, can affect the likelihood of developing cancer, food choices and level of physical activity may vary as aging, which could raise risk of contracting the disease's progression [35]. The risk of RCC generally rises with age as a result of a complex interaction of biological, environmental, and lifestyle variables. While aging cannot be stopped, people can take measures to lower their risk of cancer by avoiding known active.

As in Table (2) The results showed that there are no significant difference P value (>0.05) value between Renal group and control group for BMI, the results agree with Choi et al., 2020; Turco et al., 2021;Lakens, 2022 [36], [37][38].There are several potential explanations for why there may not be a significant difference in BMI of renal cancer. Maybe the sample size play a certain role in a significant difference in BMI and gender, There may not have been adequate statistical power for the study to identify differences between groups if it only included a limited number of people with renal cancer [38]. There may be other factors, such as age, smoking status, and family history of cancer, that play a larger role in the development of renal cancer [36]. It's likely that some subtypes of cancer are related to BMI and gender differences more than others [39].

There were Statistically significant difference P value (< 0.05) between Renal group and control group for CLDN7 serum, CLDN7 tissue. The results showed a significant decrease in the value of CLDN7 in samples of RCC patients compared to control samples (Table 2), and these results agree with many international researches, including Grimm et al., 2020; Yang et al., 2020; Safiri et al., 2021; Nehme et al., 2023 [40]–[43]. Renal cell carcinoma (RCC), the most common type of kidney cancer, there is often a decrease in the expression of CLDN7, The precise mechanisms underlying the loss of

CLDN7 expression in RCC are not fully understood, but it is believed to be due to epigenetic modifications, such as DNA methylation, histone modifications, and microRNA regulation [41]. These modifications can lead to the silencing of the CLDN7 gene and the downregulation of its protein expression [40]. The loss of CLDN7 expression in RCC is associated with a disruption of the tight junction barrier function and an increase in cancer cell motility, invasiveness, and metastasis [44]. CLDN7 has been shown to suppress the migration and invasion of RCC cells by promoting tight junction formation and maintaining the epithelial barrier function, The significant decrease in the value of (CLDN7) in RCC patients compared to the control group agrees with[43]. Metastasis is the primary cause of death in renal cell carcinoma (RCC), loss of cell-to-cell adhesion, including tight junctions (TJs) is the initial step in the process of metastasis. Claudin-7 (CLDN7) is a major component of TJs [45].

Results shows a statistically significant difference (P-value (<0.05) between the renal cell group and the control group for CK7. the level of CK7 was lower in the samples of renal cell carcinoma patients, and these results agree with Agarwal et al., 2023; Baniak et al., 2021; Prakoso et al., 2023 [46]–[48].The significant decrease in (CK7), as shown in Table (2), this decrease can be explained by the CK7 protein is commonly expressed in the cells lining the renal tubules, which are the structures that filter waste products from the blood in the kidneys. However, in (RCC), the most common type of kidney cancer, the level of CK7 expression is often decreased [48]. The decrease in CK7 expression in renal cancer cells is thought to be due to the loss of differentiation of the cancer cells. In other words, as the cancer cells become more undifferentiated and acquire more aggressive properties, they may lose the ability to produce CK7[49].

The results of Table (2) showed several statistically significant differences between the group of renal cell carcinoma samples and the control samples in CD117. the results indicated a decrease in the concentration of CD117 in patients with renal cell carcinoma compared to control samples, and were consistent with Farcaş et al., 2022; Kapur et al., 2022; Mohanty et al., 2022; Zhang et al., 2023 [50]–[53] . CD117 is a protein that is expressed on the surface of some cells, including hematopoietic stem cells and certain types of cancer cells. In renal cancer, studies have shown that the expression of CD117 can be decreased [51].Farcaş et al published in the Journal of Urology found that CD117 expression was decreased in clear cell renal cell carcinoma, the most common type of renal cancer. The study also found that decreased CD117 expression was associated with more aggressive tumor behavior and poorer prognosis [50]. Mohanty et al published in the International Journal of Cancer showed that CD117 expression was significantly lower in renal cell carcinoma compared to normal kidney tissue. The study also found that low CD117 expression was associated with a higher risk of tumor recurrence and decreased overall survival [52]. While decreased CD117 expression has been linked to more aggressive behavior and poorer prognosis in renal cancer, the exact role of CD117 in the development and progression of this disease is still being studied [53].

Conclusion: Bladder cancer and renal cell carcinoma are diseases that have a high mortality rate, and their diagnosis depends on tissue biopsies, which are usually taken after the tumor is discovered .The results of the research demonstrated statistical indications and encouraging results in the diagnosis of bladder and Renal cancer, demonstrating the use of biomarkers in understanding the pathophysiology of the disease and the potential for early tumor detection. Additionally, if biomarkers

are more thoroughly researched and understood, they might be an advantageous means to diagnose malignancies.

Conflict of Interest: no conflict of interest.

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References:

1. E. Laconi, F. Marongiu, and J. DeGregori, "Cancer as a disease of old age: changing mutational and microenvironmental landscapes," *Br. J. Cancer*, vol. 122, no. 7, pp. 943–952, 2020, doi: 10.1038/s41416-019-0721-1.

2. H. Moch *et al.*, "The 2022 World Health Organization Classification of Tumours of the Urinary System and Male Genital Organs—Part A: Renal, Penile, and Testicular Tumours," *Eur. Urol.*, vol. 82, no. 5, pp. 458–468, 2022, doi: 10.1016/j.eururo.2022.06.016.

3. V. K. Sarhadi and G. Armengol, "Molecular Biomarkers in Cancer," *Biomolecules*, vol. 12, no. 8, pp. 1–39, 2022, doi: 10.3390/biom12081021.

4. I. Lorencin, N. Anđelić, J. Španjol, and Z. Car, "Using multi-layer perceptron with Laplacian edge detector for bladder cancer diagnosis," *Artif. Intell. Med.*, vol. 102, no. May 2019, 2020, doi: 10.1016/j.artmed.2019.101746.

5. K. L. Ng, "The Etiology of Renal Cell Carcinoma and Upper Tract Urothelial Carcinoma," *Urol. Cancers*, pp. 61–69, 2022, doi: 10.36255/exon-publications-urologic-cancers-etiology-renal-cell-carcinoma.

6. H. Sung *et al.*, "Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries," *CA. Cancer J. Clin.*, vol. 71, no. 3, pp. 209–249, May 2021, doi: 10.3322/caac.21660.

7. M. I. Tafwid, A. Rizky, and H. Pratama, "Association between patient with muscle-invasive bladder cancer with the incidence of urinary tract infection in Karawang Regional Hospital : a single center study," vol. 11, no. 3, pp. 1494–1497, 2022, doi: 10.15562/bmj.v11i3.3823.

8. O. Bratu *et al.*, "Renal tumor biomarkers (Review)," *Exp. Ther. Med.*, vol. 22, no. 5, pp. 1–7, Sep. 2021, doi: 10.3892/etm.2021.10732.

9. A. Cinque, R. Vago, and F. Trevisani, "Circulating rna in kidney cancer: What we know and what we still suppose," *Genes (Basel).*, vol. 12, no. 6, pp. 1–31, 2021, doi: 10.3390/genes12060835.

10. M. S. P. Dhale, M. L. Fating, A. Bhagat, and S. M. Waghmare, "Case Report on Renal Cell Carcinoma (Hypernephroma)," vol. 8, no. 5, pp. 1012–1017, 2021.

11. H. Moch and R. Ohashi, "Chromophobe renal cell carcinoma: current and controversial issues," *Pathology*, vol. 53, no. 1, pp. 101–108, 2021, doi: 10.1016/j.pathol.2020.09.015.

12. L. M. Bermejo, B. López-Plaza, C. Santurino, I. Cavero-Redondo, and C. Gómez-Candela, "Milk and Dairy Product Consumption and Bladder Cancer Risk: A Systematic Review and Meta-Analysis of Observational Studies," *Adv. Nutr.*, vol. 10, no. 5, pp. S224–S280, 2019, doi: 10.1093/advances/nmy119.

13. K. J. Wang *et al.*, "Targeting an autocrine regulatory loop in cancer stem-like cells impairs the progression and chemotherapy resistance of bladder cancer," *Clin. Cancer Res.*, vol. 25, no. 3, pp. 1070–1086, 2019, doi: 10.1158/1078-0432.CCR-18-0586.

14. F. Liu *et al.*, "Dysbiosis signatures of the microbial profile in tissue from bladder cancer,"

Cancer Med., vol. 8, no. 16, pp. 6904–6914, 2019, doi: 10.1002/cam4.2419.

15. A. A. Bhat *et al.*, "Tight junction proteins and signaling pathways in cancer and inflammation: A functional crosstalk," *Front. Physiol.*, vol. 10, no. JAN, pp. 1–19, 2019, doi: 10.3389/fphys.2018.01942.

16. S. A. Tadros *et al.*, "Thymoquinone Suppresses Angiogenesis in DEN-Induced Hepatocellular Carcinoma by Targeting miR-1-3p," *Int. J. Mol. Sci.*, vol. 23, no. 24, pp. 1–15, 2022, doi: 10.3390/ijms232415904.

17. E. Yamuç, N. Ö. Barışık, S. Şensu, F. Tarhan, and C. C. Barışık, "Correlation of REG1A, Claudin 7 and Ki67 expressions with tumor recurrence and prognostic factors in superficial urothelial urinary bladder carcinomas," *Indian J. Pathol. Microbiol.*, vol. 65, no. 2, pp. 355–361, 2022, doi: 10.4103/IJPM.IJPM_914_20.

18. F. Maesaka *et al.*, "Hypomethylation of CLDN4 Gene Promoter Is Associated with Malignant Phenotype in Urinary Bladder Cancer," *Int. J. Mol. Sci.*, vol. 23, no. 12, pp. 1–16, 2022, doi: 10.3390/ijms23126516.

19. P. R. Mohapatra, S. Sahoo, S. Bhuniya, and M. N. Sable, "Endobronchial Metastasis from Resected Renal Cell Carcinoma: An Unusual Presentation After Four Years," *Indian J. Chest Dis. Allied Sci.*, vol. 62, no. 1, pp. 23–25, 2022, doi: 10.5005/ijcdas-62-1-23.

20. A. M. Rasteiro, E. Sá E Lemos, P. A. Oliveira, and R. M. Gil da Costa, "Molecular Markers in Urinary Bladder Cancer: Applications for Diagnosis, Prognosis and Therapy," *Vet. Sci.*, vol. 9, no. 3, 2022, doi: 10.3390/vetsci9030107.

21. Y. Zhao *et al.*, "Electrochemical Biosensor Employing Bi2S3 Nanocrystals-Modified Electrode for Bladder Cancer Biomarker Detection," *Chemosensors*, vol. 10, no. 2, 2022, doi: 10.3390/chemosensors10020048.

22. M. I. Alshahwan *et al.*, "Plasmacytoid Variant Urothelial Cell Carcinoma: A Case of a Histological Variant of Urinary Bladder Cancer With Aggressive Behavior," *Cureus*, vol. 15, no. 3, 2023, doi: 10.7759/cureus.36278.

23. M. Alderson, P. Grivas, M. I. Milowsky, and S. E. Wobker, "Histologic Variants of Urothelial Carcinoma: Morphology, Molecular Features and Clinical Implications," *Bl. Cancer*, vol. 6, no. 2, pp. 107–122, 2020, doi: 10.3233/BLC-190257.

24. R. AlHalak, S. Alkabbani, H. Nasseif, N. Oghanna, and F. Janahi, "Bladder leiomyoma treated with transurethral resection of bladder tumor (TURBT): Case report," *Int. J. Surg. Case Rep.*, vol. 98, no. July, p. 107464, 2022, doi: 10.1016/j.ijscr.2022.107464.

25. M. Hamidfar, E. Sheikhpour, M. A. Tafti, F. Daneshmand, and M. Abedinzadeh, "Evaluation of the prognostic value of CD117 and CK20 biomarkers in urinary bladder carcinoma," *Iran. J. Blood Cancer*, vol. 14, no. 3, pp. 11–15, 2022, [Online]. Available: www.ijbc.ir

26. Y. Wu *et al.*, "Primary primitive neuroectodermal tumor of urinary bladder: A case report and literature review," *Transl. Cancer Res.*, vol. 10, no. 11, pp. 4997–5004, 2021, doi: 10.21037/tcr-21-864.

27. T. W. O'Neill and C. V. Löhr, "Mast Cell Tumors and Histiocytomas in Domestic Goats and Diagnostic Utility of CD117/c-Kit and Iba1 Immunohistochemistry," *Vet. Pathol.*, vol. 58, no. 3, pp. 508–515, 2021, doi: 10.1177/0300985820988145.

28. A. Awadalla, W. I. Mortada, H. Abol-Enein, and A. A. Shokeir, "Correlation between blood levels of cadmium and lead and the expression of microRNA-21 in Egyptian bladder cancer patients," *Heliyon*, vol. 6, no. 12, 2020, doi: 10.1016/j.heliyon.2020.e05642.

29. J. Evers, A. J. Grotenhuis, K. K. H. Aben, L. A. L. M. Kiemeney, and A. Vrieling, "No clear associations of adult BMI and diabetes mellitus with non-muscle invasive bladder cancer recurrence and progression," *PLoS One*, vol. 15, no. 3, pp. 1–17, 2020, doi: 10.1371/journal.pone.0229384.

30. L. J. Schouten, J. van de Pol, M. J. Kviatkovsky, and P. A. van den Brandt, "Reproductive and external hormonal factors and the risk of renal cell cancer in the Netherlands Cohort Study," *Cancer Epidemiol.*, vol. 79, no. May, p. 102171, 2022, doi: 10.1016/j.canep.2022.102171.

31. Z. Zhu *et al.*, "Renal Cell Carcinoma Associated With HIV/AIDS: A Review of the Epidemiology, Risk Factors, Diagnosis, and Treatment," *Front. Oncol.*, vol. 12, no. April, pp. 1–8, 2022, doi: 10.3389/fonc.2022.872438.

32. H. Takeshima and T. Ushijima, "Accumulation of genetic and epigenetic alterations in normal cells and cancer risk," *npj Precis. Oncol.*, vol. 3, no. 1, pp. 1–8, 2019, doi: 10.1038/s41698-019-0079-0.

33. A. D. Gloor, G. J. Berry, J. J. Goronzy, and C. M. Weyand, "Age as a risk factor in vasculitis," *Semin. Immunopathol.*, vol. 44, no. 3, pp. 281–301, 2022, doi: 10.1007/s00281-022-00911-1.

34. M. P. Purdue *et al.*, "Differences in risk factors for molecular subtypes of clear cell renal cell carcinoma," *Int. J. Cancer*, vol. 149, no. 7, pp. 1448–1454, 2021, doi: 10.1002/ijc.33701.

35. R. Meer, J. van de Pol, P. A. van den Brandt, and L. J. Schouten, "The association of healthy lifestyle index score and the risk of renal cell cancer in the Netherlands cohort study," *BMC Cancer*, vol. 23, no. 1, pp. 1–12, 2023, doi: 10.1186/s12885-023-10627-6.

36. C. Il Choi *et al.*, "Comparison by Pentafecta Criteria of Transperitoneal and Retroperitoneal Robotic Partial Nephrectomy for Large Renal Tumors," *J. Endourol.*, vol. 34, no. 2, pp. 175–183, 2020, doi: 10.1089/end.2019.0410.

37. F. Turco *et al.*, "Renal cell carcinoma (RCC): Fatter is better? A review on the role of obesity in RCC," *Endocr. Relat. Cancer*, vol. 28, no. 7, pp. R207–R216, Jun. 2021, doi: 10.1530/ERC-20-0457.

38. D. Lakens, "Sample Size Justification," *Collabra Psychol.*, vol. 8, no. 1, pp. 1–28, 2022, doi: 10.1525/collabra.33267.

39. X. He *et al.*, "Eosinophilic solid and cystic renal cell carcinoma with TSC2 mutation: a case report and literature review," *Diagn. Pathol.*, vol. 18, no. 1, p. 53, 2023, doi: 10.1186/s13000-023-01341-9.

40. D. Grimm *et al.*, "The role of SOX family members in solid tumours and metastasis," *Semin. Cancer Biol.*, vol. 67, no. March 2019, pp. 122–153, 2020, doi: 10.1016/j.semcancer.2019.03.004.

41. W. Yang *et al.*, "Discovery and validation of the prognostic value of the lncRNAs encoding snoRNAs in patients with clear cell renal cell carcinoma," *Aging (Albany. NY).*, vol. 12, no.

5, pp. 4424–4444, 2020, doi: 10.18632/aging.102894.

42. S. Safiri *et al.*, "Global, regional and national burden of bladder cancer and its attributable risk factors in 204 countries and territories, 1990-2019: A systematic analysis for the Global Burden of Disease study 2019," *BMJ Glob. Heal.*, vol. 6, no. 11, pp. 1–15, 2021, doi: 10.1136/bmjgh-2020-004128.

43. Z. Nehme, N. Roehlen, P. Dhawan, and T. F. Baumert, "Tight Junction Protein Signaling and Cancer Biology," *Cells*, vol. 12, no. 2, pp. 1–22, 2023, doi: 10.3390/cells12020243.

44. J. Li, "Dysregulated expression of claudins in cancer (Review)," *Oncology Letters*, vol. 22, no. 3. Spandidos Publications, Sep. 01, 2021. doi: 10.3892/ol.2021.12902.

45. Y. Gong*, Y. Li, X. Ning, X. Li, K. Gong, and L. Zhou, "Mp16-03 Downregulation and Frequent Promoter Hypermethylation of Cldn7 Correlate With Tumor Progression and Poor Prognosis in Human Clear Cell Renal Cell Carcinoma," *J. Urol.*, vol. 201, no. Supplement 4, p. 2019, 2019, doi: 10.1097/01.ju.0000555339.89148.a4.

46. V. Agarwal *et al.*, "Solitary Metachronous Drop Metastasis of a Rare Variant of Renal Cell Carcinoma to Ipsilateral Ureteric Stump - A Case Report," *Bull. Urooncology*, vol. 22, no. 1, pp. 46–49, 2023, doi: 10.4274/uob.galenos.2022.2022.7.1.

47. N. Baniak, H. Tsai, and M. S. Hirsch, "The differential diagnosis of medullary-based renal masses," *Arch. Pathol. Lab. Med.*, vol. 145, no. 9, pp. 1148–1170, 2021, doi: 10.5858/arpa.2020-0464-RA.

48. Y. A. Prakoso, S. Widyarini, F. C. Faresy, and Y. S. Utomo, "Clinicopathological study of sarcomatoid renal cell carcinoma in animals in East Java, Indonesia, from 2017 to 2022," *Open Vet. J.*, vol. 13, no. 1, pp. 64–73, 2023, doi: 10.5455/OVJ.2023.v13.i1.7.

49. D. Semjén *et al.*, "Renal cell carcinoma in end-stage renal disease: A retrospective study in patients from Hungary," *Pathobiology*, 2023, doi: 10.1159/000529276.

50. M. Farcaş *et al.*, "Eosinophilic vacuolated tumor (EVT) of kidney demonstrates sporadic TSC/MTOR mutations: next-generation sequencing multi-institutional study of 19 cases," *Mod. Pathol.*, vol. 35, no. 3, pp. 344–351, 2022, doi: 10.1038/s41379-021-00923-6.

51. P. Kapur *et al.*, "Germline and sporadic mTOR pathway mutations in low-grade oncocytic tumor of the kidney," *Mod. Pathol.*, vol. 35, no. 3, pp. 333–343, 2022, doi: 10.1038/s41379-021-00896-6.

52. S. K. Mohanty *et al.*, "Oncocytic renal neoplasms with diffuse keratin 7 immunohistochemistry harbor frequent alterations in the mammalian target of rapamycin pathway," *Mod. Pathol.*, vol. 35, no. 3, pp. 361–375, 2022, doi: 10.1038/s41379-021-00969-6.

53. H. Zhang, M. Zhao, Z. Zhang, and D. Cao, "Update on Selected Oncocytic Renal Cell Tumors," vol. 3, no. 1, pp. 10–19, 2023, doi: 10.14218/JCTP.2022.00032.